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Purification and characterization of recombinant Bet v I, the major birch pollen allergen. Immunological equivalence to natural Bet v I.

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Pollen from trees of the order Fagales (e.g. birch, alder, hazel, oak, and hornbeam) are a major cause of Type I allergies observed in early spring. Previously, we reported the cloning and sequencing of Bet v I, the major birch pollen allergen, which showed high sequence similarities to a family of plant pathogen-activated genes (Breiteneder, H., Pettenburger, K., Bito, A., Valenta, R., Kraft, D., Rumpold, H., Scheiner, O., and Breitenbach, M. (1989) EMBO J. 8, 1935-1938). Here, we present the results on the expression, purification, and characterization of recombinant Bet v I produced in Escherichia coli as fusion and non-fusion protein, respectively. The purified recombinant proteins were analyzed to verify purity and structural integrity, and their immunological properties were compared to those of Bet v I isolated from birch pollen (natural Bet v I). Immunoblot analyses showed that the recombinant proteins are specifically recognized by monoclonal antibodies raised against natural Bet v I as well as by IgE from birch pollen-allergic patients. However, enzyme-linked immunosorbent assays revealed a decreased IgE-binding activity of the recombinant fusion Bet v I compared to the non-fusion and natural Bet v I proteins, which probably results from conformational changes due to the fusion tail. Recombinant non-fusion Bet v I was equivalent to natural Bet v I with respect to IgE-binding properties, the ability to induce in vitro proliferation of allergen-specific T-cell clones, and the ability to release histamine from basophils derived from birch pollen-allergic patients.

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